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The effects of androstenediol and dehydroepiandrosterone on the course and cytokine profile of tuberculosis in BALB/c mice

R. HERNANDEZ-PANDO,* M. DE LA LUZ STREBER,* H. OROZCO,* K. ARRIAGA,* L. PAVON,* S. A. AL-NAKHLI† & G. A. W. ROOK† **Experimental Pathology Laboratory, Department of Pathology, Instituto Nacional de la Nutricion 'Salvador Zubiran', Mexico City, Mexico, and †Department of Bacteriology, UCL Medical School, London, UK*

SUMMARY

Immunity to *Mycobacterium tuberculosis* requires a T helper 1 (Th1) cytokine balance accompanied by tumour necrosis factor-α (TNF-α), and activated macrophages. These facets of the immune response are sensitive to suppression by glucocorticoids (GC), which can reactivate and exacerbate tuberculosis in man and animals. Dehydroepiandrosterone (DHEA) and its derivative, 3β,17β androstenediol (AED), are reported to have antiglucocorticoid properties *in vivo*. We therefore investigated the effects of predetermined optimal doses of these compounds, on the course of pulmonary tuberculosis in an established model in BALB/c mice in which an early phase of Th1-mediated response accompanied by adrenal hyperplasia, is followed by a switch to Th2, progressive loss of TNF-α expression and disease progression. Both compounds were protective, particularly AED which caused a fall in bacterial counts and prolonged survival. These effects correlated with the appearance within 3 days of cellular infiltrates rich in cells expressing interleukin-2 (IL-2), IL-1α and TNF-α, and with partial suppression of the switch to IL-4 producing cells that occurred in controls. AED also caused enhanced development of granulomas at 14 days, and persistence of granuloma formation to 120 days, with a corresponding suppression of areas affected by pneumonia. Much of the therapeutic effect of AED and DHEA was obtained by treating for only the first 3 weeks, which is the phase of adrenal hyperplasia. These results suggest that the ratio of GC to anti-GC steroids may play a role in the pathogenesis of tuberculosis, and further investigation could lead to novel treatment strategies.

INTRODUCTION

Dehydroepiandrosterone (DHEA) is the most abundant product of the human adrenal gland after adrenarche. In healthy young adults the adrenal secretes 10–15 mg of DHEA sulphate (DHEAS) per day and it is present in plasma at concentrations close to 4 μg/ml. Levels then fall steadily with increasing age. The sulphate is strongly bound to albumin and undergoes renal tubular reabsorption. Eventually most of it is converted to DHEA. Most DHEA circulates free but some is weakly bound to albumin. The DHEA derivative 3β, 17β androstenediol (AED) differs only in that the 17-keto of DHEA is reduced to a β-hydroxyl group.

DHEA has been shown to have antiglucocorticoid properties in a number of systems. Thus, DHEA can oppose effects of glucocorticoids (GC) on enzyme expression in the liver, and on obesity,[1] and also their effects within the immune system.[2–4] The molecular mechanisms are unclear. It does not act as a competitive antagonist of glucocorticoid (GC) receptors, and a recent hypothesis is that it operates via peroxisome proliferator-activated receptor alpha (PPARα).[5]

Modulation of the effects of GC may be important in the pathogenesis of tuberculosis. GC tend to reactivate human and murine[6,7] tuberculosis, and restraint stress reactivates latent tuberculosis in mice by a GC-dependent mechanism.[8] These effects may reflect the ability of GC to impair the ability of macrophages to control the proliferation of mycobacteria,[9] and to deviate the immune response towards a Th2 cytokine profile.[10,11] Thus, although GC are able to suppress production of type 2 cytokines by mature Th2 cells when administered at pharmacological levels (and are frequently used for this purpose in asthma and hayfever), GC cause newly recruited T cells to develop a Th2 cytokine profile.[10,11] Similarly GC can enhance Th2 activity and synergize with Th2 cytokines.[12–15]

As the anti-GC properties that have been claimed for DHEA include promotion of Th1 cytokine production from murine and human cells,[4,16] we have tested the effects of DHEA and AED in a well-characterized model of pulmonary tuberculosis in the mouse.[17,18] In this model adrenal hyperplasia occurs at the peak of the early Th1-dominated response at 2–3 weeks,[19,20] which is followed by a switch to a mixed

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Correspondence: Professor G. A. W. Rook, Department of Bacteriology, U. C. L. Medical School, Windeyer Building, 46 Cleveland St., London W1P 6DB, UK.

Th1 plus Th2 pattern.[17,20] We have documented effects of optimal doses of AED or DHEA on survival, bacterial counts and cytokine profile.

MATERIALS AND METHODS

Experimental model of tuberculosis infection in mice

All animal work was performed in conformity with the Home Office regulations in the UK, or the Local Ethical Committee for Experimentation on Animals in Mexico. The tuberculosis model has been described in detail elsewhere.[17–19] Briefly, male BALB/c mice were used at 6–8 weeks of age, and infected by direct intratracheal injection with 1×10^6 viable *M. tuberculosis* H37Rv in 100 µl of phosphate-buffered saline (PBS), as described elsewhere.[18] Infected animals were maintained in groups of five in cages fitted with microisolators. Mice were killed and exsanguinated at 1, 3, 7, 14, 21, 28, 60 and 120 days.

Assessment of colony-forming units in infected lungs

Half of each right and half of each left lung was used for colony counting, while the other halves were used for studying other parameters. Lungs were homogenized with a Polytron (Kinematica, Luzern, Switzerland) in sterile 50-ml tubes containing 3 ml of isotonic saline. Four dilutions of each homogenate were spread onto duplicate plates containing Bacto Middlebrook 7H10 agar (Difco Lab code 0627-17-4, Difco Labs, Detroit, MI) enriched with OADC also from Difco code 07-22-64-0). The time for incubation was 21 days. Two animals were killed at each time point, in two different experiments, so the data points are the means of four animals.

Determining the appropriate doses of DHEA and its derivative AED

DHEA and its derivative AED were both obtained from Sigma (Poole, Dorset, UK) and dissolved in ultrapure olive oil (Sigma), and administered s.c. in a volume of 50 µl. There are wide discrepancies in the doses used in mice by different authors, ranging from 1 gm/kg,[21] to 500 µg/kg.[22] Therefore the correct dose range for these steroids in mice was obtained by determining the dose that optimally opposed the ability of corticosterone to cause involution of the thymus. This assay was based on the observations of Blauer *et al.*[2] Briefly, the doses of AED or DHEA to be tested were injected s.c. into five mice on three consecutive days. Aetiocholanolone (3α-hydroxy-5β-androstan-17-one), a derivative of DHEA that is inactive in this assay, was used as a control. The last dose was given at the same time as 1·6 mg of corticosterone, and the thymuses were weighed 24 hr later. This dose of corticosterone had been shown previously to cause a 50% reduction in thymus weight. The thymus protection assay suggested a dose range of 1–20 µg/mouse/day following this three-dose regimen. Then further dose-response studies were carried out over a narrower dose range, using three s.c. doses per week (i.e. Monday, Wednesday and Friday, rather than three consecutive doses) in the tuberculosis model itself, as described in the results.

Preparation of tissue for histology, morphometry and immunohistochemistry

For histological study, lungs were prepared as described.[18] The following five parameters were then measured on haematoxylin and eosin-stained parasaggital sections in μ^2 with a Zidas Zeiss image analysis system (Welwyn Garden City, Herts, UK): area of peribronchial infiltration, area of perivascular infiltration, area of granuloma, area of interstitial inflammation, percentage of lung affected by peumonia. Data are expressed as the mean of 4–6 animals ± SD.

For immunohistochemistry lung sections were mounted on silane-coated slides, deparaffinized, and the endogenous peroxidase quenched with 0·03% H_2O_2 in absolute methanol. Lung sections were incubated overnight at 4° with biotin-labelled polyclonal goat antibodies against IL-2 or IL-4 (R&D Systems, Minneapolis, MI), or with polyclonal rabbit antibodies to TNF-α or IL-1α (Genzyme, Cambridge, MA) diluted 1/50 in PBS. Bound antibodies were detected with avidin-biotin peroxidase (Vector, Burlinghame, CA) and counterstained with haematoxylin. For quantitation of immunohistochemistry, three random fields of each pulmonary compartment were evaluated at ×400 magnification in four to six mice, and expressed as the mean ± SD. Immunohistochemically positive and negative cells located in the alveolar-capillary interstitium, perivascular and peribronchial inflammation, granulomas and pneumonic areas were counted and the number of positive cells was expressed as a percentage of the total number of cells present.

Measurement of delayed hypersensitivity (DTH)

The antigen and procedure are described in detail elsewhere.[18]

Statistical analysis

A Student's *t*-test was used for analysis of differences between groups. A P value of 0·05 or less was considered significant.

RESULTS

Selection of appropriate dose regimens for DHEA and AED

Thymic involution was induced with a single injection of corticosterone (1·6 mg), and the ability of various doses of DHEA, AED, or aetiocholanolone to block this involution was determined as described in the Materials and Methods. Figure 1 shows pooled data from four experiments normalized by calculating the percentage preservation of thymic weight. Appropriate doses lay in the range of 1–20 µg. Aetiocholanolone did not protect the thymus.

In order to further verify these dose ranges, which are lower than used by other authors working in infection models, further dose-response studies were carried out over a narrower dose range in the tuberculosis model itself, using three s.c. doses per week (i.e. Monday, Wednesday and Friday) rather than three consecutive doses. Histological changes and maintenance of DTH responsiveness were used as endpoints. Data from these pilot experiments are not shown since the findings were then repeated and expanded in the studies reported below. Selected doses were 50 µg three times per week for DHEA and 12 µg three times per week for AED. This dose regimen was started on the day of infection, and continued for 3 weeks only, or for the entire duration of the experiments (120 days). Control mice received olive oil only on the same days.

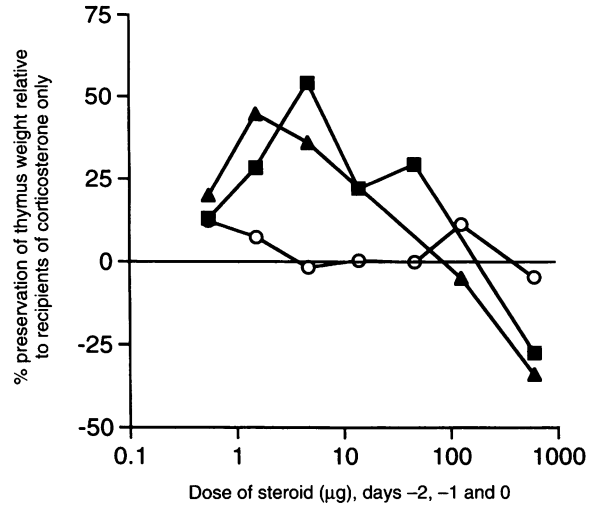

Figure 1. The use of the thymus protection assay to optimize doses of DHEA (■) and AED (▲). Control animals (○) received aetiocholanolone, which is inactive in this assay.

Effects of AED and DHEA on survival and bacterial numbers after infection with *M. tuberculosis*

Recovery of the inoculum was complete, as anticipated following direct intratracheal injection in a species that cannot cough. Colony-forming units (CFU) in the lungs rose steadily in the control mice given olive oil only, whereas they fell in animals treated continuously with AED 12 μg three times per week (Fig. 2). Treatment with DHEA caused a plateau or slight fall in CFU.

Survival curves were not a major part of this study. Ten control mice and 10 AED-treated mice were left undisturbed and their survival is recorded as an insert in Fig. 2. Fisher's exact test on mortality rates at 60 days and 120 days gives P values of 0·07 and 0·089, respectively. Death, as reported previously, was because of pneumonitis, and filling of the air spaces with cells which occurs in this model when the Th2 component of the response becomes prominent.[18]

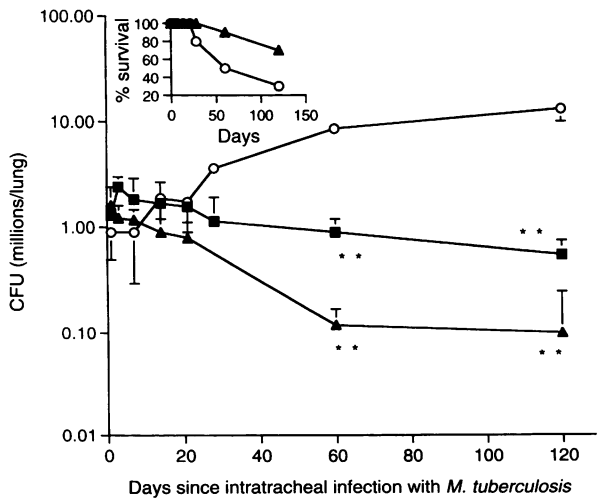

Figure 2. Colony-forming units in the lungs of BALB/c mice killed at intervals after intratracheal infection with 10^6 *Mycobacterium tuberculosis* H37Rv. In this and in all subsequent figures open circles represent controls, filled triangles represent AED-treated, and filled squares represent DHEA-treated mice. The insert shows survival curves for control and AED-treated animals only.

The effects of DHEA and AED on lung pathology in tuberculous mice

Morphometric analysis of the zones of inflammation in mice on the continuous treatment regimen, and appropriate controls, revealed that both steroids caused enhancement of the early inflammatory reaction, with diminution of the late phase of pneumonia (Fig. 3). Some peribronchial inflammation was seen in all animals by day 1 (Fig. 3a). However, in the steroid-treated animals there was a sharp peak of further inflammation on day 3, which had subsided by day 14. A similar enhancement of inflammatory infiltration was seen in the interstitial and perivascular compartments (data not shown).

No granuloma formation was seen in any mice before day 14, but at that time it was significantly greater in the steroid-treated groups (Fig. 3b). In the mice treated with AED there was also significantly more granuloma at days 60 and 120 ($P<0·005$ relative to controls at both time points).

No pneumonia was seen in either treated group until day 60 (Fig. 3c), whereas it was already present at the day 28 timepoint in the controls. The percentage of the lung involved in this process remained low in the treated animals, particularly

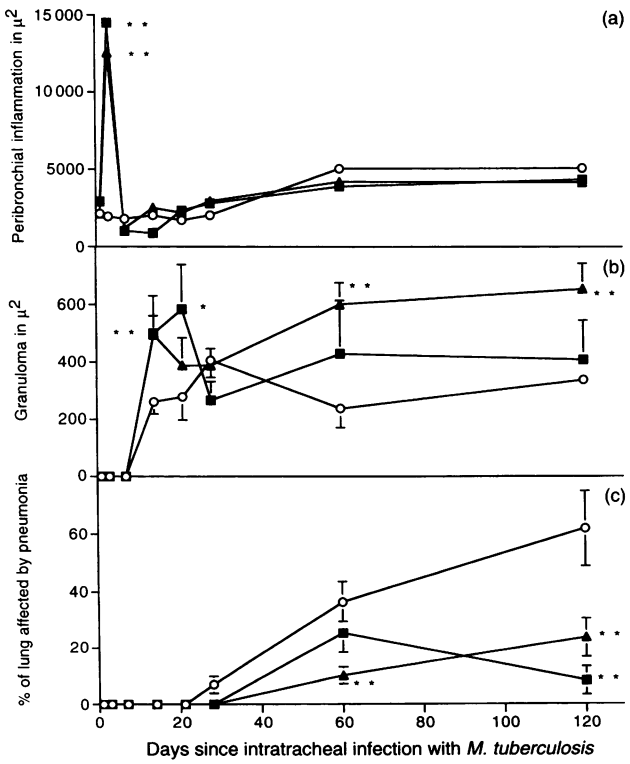

Figure 3. Morphometric analysis of infected lungs. (a) area of peribronchial infiltration. (b) Area of granuloma. (c) Percentage of the lung affected by pneumonia. Both steroids caused an early peak (3 days) of peribronchial involvement, and they enhanced granuloma formation between days 14 and 21, and again in the late stages (days 60 and 120), while inhibiting pneumonia. (*$P<0·025$; **$P<0·005$.) ○, control animals; ▲, treated with AED; ■, treated with DHEA.

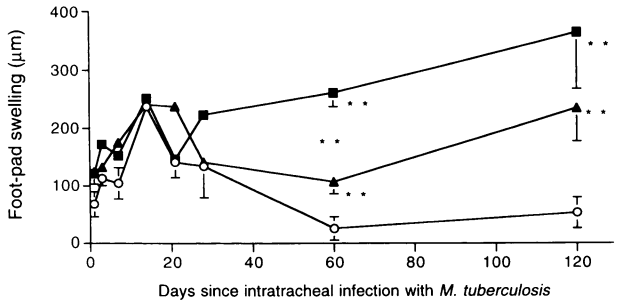

Figure 4. Delayed hypersensitivity responses to soluble antigens of *M. tuberculosis* in tuberculous mice. Swelling was measured 24 hr after challenge. Both steroids enhance DTH responses at days 60 and 120. At day 60 DHEA enhanced significantly more than AED ($P<0{\cdot}005$). (*$P<0{\cdot}025$; **$P<0{\cdot}005$.) ○, control animals; ▲, treated with AED; ■, treated with DHEA.

in those treated with AED ($P<0{\cdot}005$ relative to controls at day 60 and day 120).

This reversal of the ratio of granuloma to pneumonia in the AED-treated mice is in agreement with the changes in CFU and survival seen in Fig. 2.

The effect of DHEA and AED on DTH responses

While it is clear that granuloma correlates with resistance in this model, and pneumonia with rapid progression,[18] the role of DTH is unclear. The DTH responsiveness of control mice peaked at day 14 and then declined. The responses of the steroid-treated groups were similar until day 60, when there was strikingly greater responsiveness in all treated mice, particularly in those treated with DHEA (Fig. 4). The effect of DHEA was significantly greater than the effect of AED on day 60 ($P<0{\cdot}005$). Thus by this parameter DHEA was more effective than AED, whereas by the more directly relevant parameters of CFU (Fig. 2) and histology (Fig. 3), it was not.

Immunohistochemical analysis of expression of IL-2 and IL4

We have shown previously that in this model of pulmonary tuberculosis in BALB/c mice, susceptibility to disease[18] and disease progression[17] correlate with the presence of IL-4$^+$ cells. In order to ascertain whether the beneficial effects of AED and DHEA were related to changes in Th1/Th2 balance, immunohistochemical analysis of cells staining positive for IL-2 and IL-4 were performed (Fig. 5). Interferon-γ (IFN-γ) was not used as a Th1 correlate because it is not T-cell specific. The animals were from the groups undergoing continuous treatment with steroid in olive oil, or olive oil alone.

The day 3 peak of inflammatory infiltration induced by AED and DHEA (Fig. 3a) was reflected in a significant increase in IL-2$^+$ cells in the perivascular (Fig. 5a) peribronchial, and interstitial zones (data not shown). The percentage IL-2$^+$ cells caught up in control animals between days 14 and 21, and then declined again, so that from day 28 there were significantly more IL-2$^+$ cells in the steroid-treated animals (Fig. 5a). The same was true in the other inflammatory zones (data not shown).

The reverse was true of IL-4$^+$ cells in the later stages of the infection (Fig. 5b). IL-4$^+$ cells were present at a lower

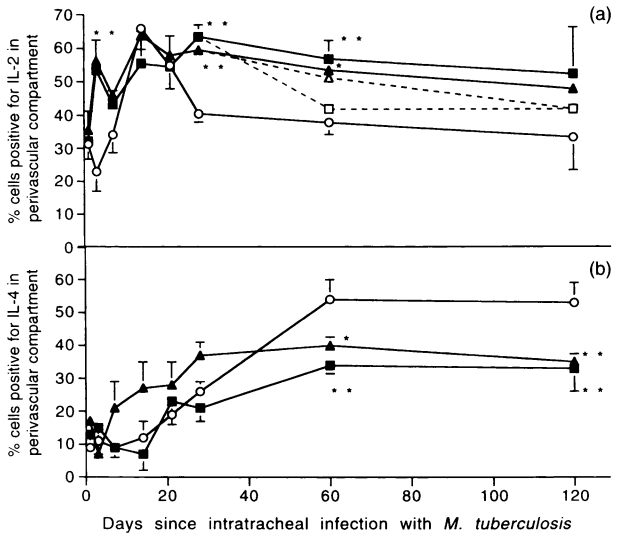

Figure 5. Immunocytochemical analysis of (a) IL-2 positive and (b) IL-4-positive cells in the perivascular compartment of the lungs of BALB/c mice at intervals after infection into the trachea with *M. tuberculosis* H37Rv. Data are the means ± SD of 9–18 random fields (three fields from each of four to six mice). Treatment with either steroid caused an early (day 3) peak of IL-2-positive cells, and also increased the percentage of IL-2-positive cells at days 28 and 60. Similarly both steroids caused a decreased percentage of IL-4-positive cells on days 60 and 120. (*$P<0{\cdot}025$; **$P<0{\cdot}005$.) ○, control animals; ▲, treated with AED; ■, treated with DHEA.

percentage in the perivascular compartment of animals treated with either AED or DHEA on days 60 or 120. Examples of low IL-4 positivity and high IL-2 positivity in granulomas after 120 days of treatment with DHEA are shown in Fig. 6 (C,D). The results were similar in the other lung zones and results for the peribronchial, interstitial and granulomatous compartments on days 60 and 120 are shown in Fig. 7.

Further support for the view that the steroids were biasing the response towards Th1 and away from Th2 came from studies of immunoglobulin G (IgG) antibody by enzyme-linked immunosorbent assay (ELISA) in microtitre plates coated with soluble antigen from *M. tuberculosis*. Little antibody was seen until day 28. Then IgG1 levels rose in control animals, but IgG2a antibody levels stayed low. In contrast, treated animals had little antibody by day 28, and by day 60 the response was dominated by IgG2a (data not shown).

The effect of therapy on IL-4 and IL-2 for the first 3 weeks only, compared with continuous therapy

In this model there is adrenal hyperplasia for the first 3 weeks,[19,20] accompanied by increased release of GC during this period (unpublished observations). This fact, together with the striking changes in early events caused by the two steroids such as enhanced inflammatory infiltration on day 3 Fig. 3(a), and enhanced granuloma formation on day 14 (Fig. 3b), suggested that it would be important to discover whether continued treatment with the steroids after the first 3 weeks was neccessary. The dashed lines in Fig. 5(a) show that for IL-2, terminating treatment at 3 weeks resulted in diminished effects, significant at day 60 for AED only.

The day 60 and day 120 data for IL-4 expression after

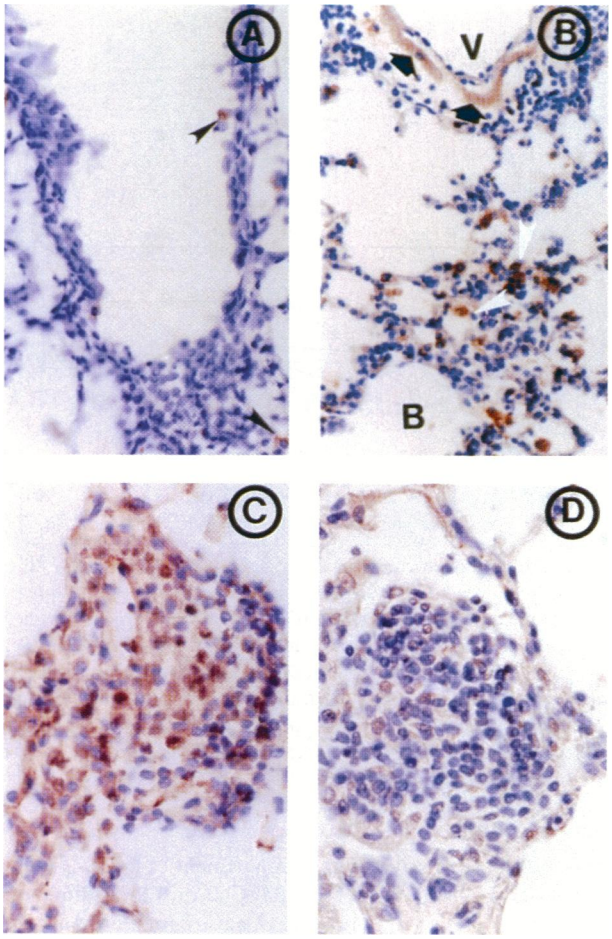

Figure 6. Representative histopathology and immunohistochemistry at different times during the evolution of experimental pulmonary tuberculosis in control mice and in mice treated with AED or DHEA. (A) Light micrograph of control mouse lung 3 days after intratracheal infection. There is slight inflammatory infiltration in the bronchial wall with a few IL-1 immunostained macrophages (arrowheads). (B) Increased inflammatory response in the interstitium (white arrowhead) and in the blood vessel (**V**) and distal bronchiolar wall (**B**), with numerous IL-1 immunostained macrophages after 3 days of infection in an animal that had received two subcutaneous injections of AED. Note that smooth muscle cells in the blood vessel wall also show immunostaining for IL-1 (arrows). (C) This part shows the large number of IL-2 immunostained cells compared with the very small numbers of IL-4 immunostained cells (D), seen in a lung granuloma 120 days after infection, from mice that had been treated with DHEA throughout.

treatment for only 3 weeks, are compared with controls and with continuous treatment in Fig. 7. This provides data for peribronchial, interstitial and granulomatous compartments not shown in the line graphs in Fig. 5. Treatment with AED for the first 3 weeks did not diminish IL-4 expression in the granulomatous areas at either 60 days or 120 days. However, the effect on IL-4 expression in the peribronchial areas did persist significantly at these late timepoints ($P<0{\cdot}005$ for day 60, $P<0{\cdot}025$ for day 120). The effect in the interstitial areas was significant at 60 days ($P<0{\cdot}005$) but not at 120 days.

Thus some, but not all, of the effect can be reproduced by treating for the first 3 weeks only.

Immunohistochemical analysis of expression of IL-1α and TNF-α

Tumour necrosis factor-α is essential for immunity to tuberculosis in mice,[23] but its expression declines in the late stages of the infection in the model used here.[17,18] Therefore, in order to discover whether the beneficial effects of AED or DHEA could be related to these mediators, the percentage of cells expressing IL-1α or TNF-α was assessed by immunohistochemistry in mice receiving continuous steroid treatment.

Figure 8 shows that the early peribronchial inflammatory infiltrate caused by AED on day 3 (shown above in Fig. 3a) was rich in cells positive for TNF-α (Fig. 8a) and IL-1α (Fig. 8b) ($P<0{\cdot}005$ for both cytokines). Examples of low IL-1 positivity at 3 days in a control lung, and increased IL-1 positivity at 3 days in an animal that had received two doses of AED, are shown in Fig. 6 (A, B). It is interesting that after two doses of AED or DHEA there was also increased expression of the pro-inflammatory cytokines in the smooth muscle cells of the bronchioles and blood vessels (Fig. 6B).

The enhanced granuloma size caused by AED at day 14 (shown above in Fig. 3b) was also rich in cells positive for both cytokines ($P<0{\cdot}005$ compared with controls), and AED significantly enhanced expression of IL-1α and TNF-α at day 120. The effects on IL-1α expression were similar for DHEA, but DHEA was less efficient at enhancing early TNF-α expression (data not shown), though the enhancement was still significant ($P<0{\cdot}025$ between days 3 and 14).

The effect of therapy on IL-1 and TNF for the first 3 weeks only, compared with continuous therapy

In view of the strong effects on early expression of IL-1α and TNF-α shown in Fig. 8, we again investigated the effect of giving the steroid for the first 3 weeks only. Figure 9 also provides examples of data generated with DHEA rather than AED, which were shown in Fig. 8.

When DHEA was given throughout, there were significantly increased numbers of cells expressing IL-1α and TNF-α on day 120, relative to controls, in all compartments. Data shown are for the perivascular and interstitial zones, and the areas of pneumonia (though these were scant in the treated animals, as shown in Fig. 3c). When treatment was given for the first 3 weeks only, there were no effects on IL-1α by 120 days, but TNF-α expression was enhanced in the interstitial and pneumonic areas at day 120, which is 100 days after cessation of the therapy.

DISCUSSION

This study has shown that at appropriate doses, DHEA or its derivative AED, are protective in a model of pulmonary tuberculosis in the mouse. This protective effect correlates with maintenance of a higher ratio of IL-2^+ to IL-4^+ cells, higher expression of IL-1α and TNF-α, and larger DTH responses. These results agree with a recent study of an influenza model.[24] Meanwhile the histology is shifted from pneumonia to granuloma. Interestingly AED was more protective, as judged by CFU, and this was associated with more prolonged enhancement of granuloma formation, and with greater enhancement of TNF-α expression in early granuloma and in peribronchial inflammatory zones. In contrast, DHEA, though less

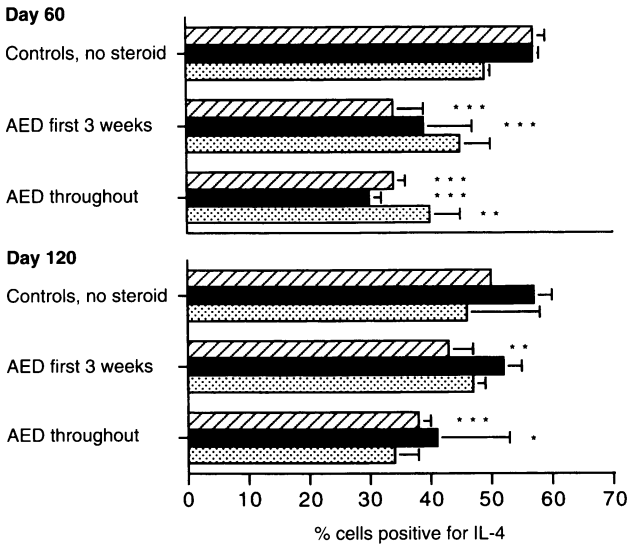

Figure 7. The effects on the percentage of cells positive for IL-4, of AED given for the first 3 weeks, compared with AED administration throughout. Data (means ± SD) are shown for days 60 and 120, in the peribronchial (hatched), interstitial (black) and granulomatous (stippled) compartments. AED decreased IL-4 expression at day 60 even when given for the first 3 weeks only, particularly in the peribronchial zones. (*$P<0{\cdot}05$; **$P<0{\cdot}025$; ***$P<0{\cdot}005$.)

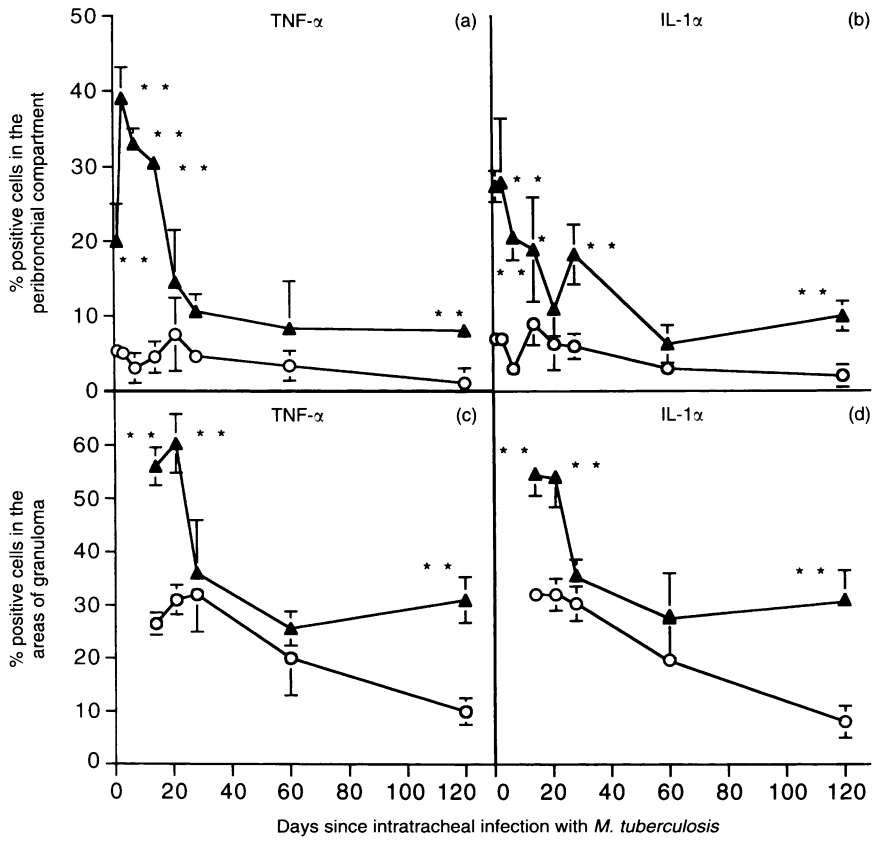

Figure 8. Immunocytochemical analysis of (a and c) TNF-α-positive and (b and d) IL-1α-positive cells in the peribronchial compartment (a and b) and granulomatous zones (c and d) of the lungs of BALB/c mice at intervals after infection into the trachea with *M. tuberculosis* H37Rv. Data are the means ± SD of 12–18 random fields (three fields from each of four to six mice). Treatment with AED caused early peaks (days 3–21) of both IL-α and TNF-α-positive cells, and also increased the percentage of positive cells at day 120. (*$P<0{\cdot}025$; **$P<0{\cdot}005$). ○, control animals; ▲, treated with AED.

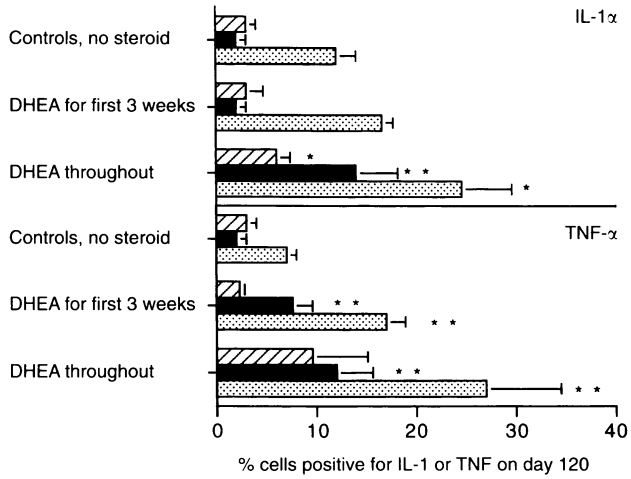

Figure 9. The effects on the percentage cells positive for IL-1α and TNF-α, of DHEA given for the first 3 weeks, compared with DHEA administration throughout. Data are shown for day 120, in the perivascular (hatched), interstitial (black) and pneumonic (stippled) compartments. DHEA increased TNF-α expression in the interstitial and pneumonic areas at day 120, even when given for the first 3 weeks only. (*$P<0.025$; **$P<0.005$.)

protective, was more potent at enhancing DTH responsiveness. This may imply a lack of correlation between DTH and protection.

The early induction of IL-2 and TNF-α-rich inflammatory infiltrates is likely to have been important, although it was not accompanied by an immediate fall in bacterial load. The effects of the steroids were significant even if no more was given after the first 3 weeks. This agrees well with the known importance of TNF-α[23] and the type 1 cytokine profile[18,25,26] in response to this infection. The changes seen at day 60 after treatment for 3 weeks may reflect the reduced bacterial loads in these animals. However, comparison of continuous treatment with treatment for only the first 3 weeks showed that administration of the steroids later in the infection is also relevant.

We have found previously that the toxicity of TNF-α in mixed Th1 + Th2 responses, such as that seen in this model, is related to the size of the Th2 component, and is minimal when Th1 is the dominant or the only detectable response present.[18,27] This may help to explain the fact that the increased TNF-α expression was associated with protection rather than increased immunopathology, as there was a simultaneously switch back to a response dominated by Th1 in the same animals.

It is possible that early administration of the antiglucocorticoid hormones DHEA or AED blocks a physiological mechanism that uses increased GC production, reflected in this model as adrenal hyperplasia,[19,20] to terminate the early Th1 response, and deviate it towards Th2. This modulation of the Th1 response after the first 3 weeks is clearly premature in BALB/c mice with pulmonary tuberculosis.

These findings have implications for human tuberculosis. However, the physiology of DHEA in rodents is clearly quite different from its physiology in man. Whereas there are about 4 μg/ml of DHEAS in adult human plasma, rat plasma contains <1 ng/ml of this hormone.[28] In mice a dose of three daily injections of 1·2 mg DHEA (i.e. 50–60 mg/kg/day) was found to stop dexamethasone from rendering peripheral lymphocytes of mice unresponsive to mitogens, or causing involution of the thymus.[2] We used this assay to determine dose for our studies, and found that doses in the μg range are more effective. Thus we have used doses more consistent with rodent DHEA levels. This may partially reflect the greater bioavailability of the steroid when dissolved in olive oil. We found that larger doses actually diminish the effect in the thymus protection assay used by Blauer and colleagues,[2] probably because of conversion to sex steroids. Gonadectomy is known to increase thymic bulk, while oestrogen and testosterone decrease it.[29]

In general, DHEA, using acceptable doses in the low μg range, appears to oppose glucocorticoids, and to promote a Th1 cytokine pattern. It has been shown to restore immune functions in aged mice, and to correct the disregulated spontaneous cytokine release seen in old animals.[22,30] It has been tested for similar properties in aged humans.[31] It also enhances production of Th1 cytokines such as IL-2 and IFN-γ.[4,32–34] This is the reverse of the effect of glucocorticoids that often enhance Th2 activity and synergize with Th2 cytokines.[12–15]

Data are more scant in man. However, a deficit in DHEA relative to cortisol can be striking in severe human tuberculosis,[35] and a fall in DHEA levels is a good marker of progression to AIDS in human immunodeficiency virus (HIV)-infected individuals.[36] In both diseases this correlates with a defect in Th1-mediated immunity, as seen in late tuberculosis in mice. DHEA also enhances IL-2 secretion from human peripheral blood T cells,[16] and we have found that DHEA or 3β,17β-androstenediol (AED) will enhance mitogen-stimulated production of IFN-γ from murine or human T cells in the range 10^{-7}–10^{-8} M, particularly when these cells are first suppressed by the addition of glucocorticoid (Al Nakhli *et al.*, in preparation).

Our findings may help to cast light on the age-related changes in susceptibility to tuberculosis, and in the *type* of disease that developes in children.[37] In children less that 5-years old, tuberculosis is common, but characterized by consolidation, and pneumonia without cavitation.[37] Levels of DHEAS are low in this age group.[38] However, children are resistant to tuberculosis between the ages of 5–10 years,[37] when there are intermediate levels of DHEAS,[38] in spite of evidence from skin testing of continuing exposure to the organism. Finally, susceptibility to tuberculosis returns at puberty, when DHEAS levels reach adult values,[38] and the pathology is characterized by cavitation and necrosis, as in the adult disease.[37] As glucocorticoid levels are constant throughout these age groups, it is possible that there is an ideal ratio of DHEA to glucocorticoid, which is only present in the 5–10-year age group. Too much DHEA may provoke tissue damage, while too little may permit a switch to Th2, as in BALB/c mice without DHEA or AED supplements. Further studies in the model reported here support this hypothesis.[39]

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